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Measurement of Chromosomal Aberrations, Sister Chromatid Exchange, *hprt* Mutations, and DNA Adducts in Peripheral Lymphocytes of Human Populations at Increased Risk for Cancer

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Using a multidisciplinary approach, we have measured various indicators of DNA damage in peripheral lymphocytes of human populations potentially at increased risk for cancer. Sister chromatid exchanges (SCE) and polycyclic aromatic hydrocarbon (PAH)–DNA adducts were evaluated in a group of firefighters; chromosomal aberrations and *hprt* mutations were evaluated in a group of cancer patients undergoing radioimmunoglobulin therapy (RIT); SCE and acrolein-modified DNA were measured in cancer chemotherapy patients and in pharmacists preparing chemotherapy prescriptions; and SCE and PAH–DNA adducts are being measured in U.S. army troops stationed in Kuwait. Our results indicate that both SCE and PAH–DNA adduct levels were not elevated in firefighters, but that other factors such as smoking status and race were risk factors for increased SCE and PAH–DNA adducts. RIT was found to increase background rates of chromosome-type aberrations and frequencies of *hprt* mutations and there was a strong correlation between levels of therapy-induced chromosome damage sustained *in vivo* and *in vitro* sensitivity to radiation-induced chromosome damage. Peripheral blood lymphocytes of cancer patients treated with cyclophosphamide showed higher levels of SCE and had a higher incidence of acrolein adducts in DNA. Lymphocytes from pharmacists preparing antineoplastic drugs were found to acquire increased *in vitro* sensitivity to SCE induction by phosphoramide mustard with increased lifetime duration of drug handling. A prospective, longitudinal study was performed to identify environmental factors that modulate genetic damage in breast cancer patients. Women with benign breast masses and no apparent disease served as controls. Mutant frequency, cloning efficiency, and chromosomal aberration frequency did not differ significantly among the three groups. The results described and the data being gathered on troops stationed in Kuwait suggest that all the methodologies described can be useful in screening human populations for mutagenic exposures.

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Introduction

Induction of DNA damage and the resulting adverse sequelae such as mutations and chromosomal rearrangements are the primary mechanisms for induction of cancer. Recent studies have shown how these events are involved in activation of dominant oncogenes and the inactivation of tumor-suppressor genes (*1*). This information permits the design of multidisciplinary approaches for monitoring human populations for exposure to carcinogenic agents. These approaches have used the collaborative efforts of a number of disciplines including epidemiology, occupational medicine, cytogenetics, immunology, molecular biology, and statistics.

This paper summarizes the results of previously published studies on groups of firefighters, pharmacists, and cancer patients. These groups have been exposed to a number of modalities including pyrolysis and combustion products, cytotoxic drugs, and radioimmunoglobulins. End points examined in these populations include chromosomal aberrations, sister chromatid exchanges, *hprt* mutations, and DNA adducts. The utility and disadvantages of each of these end points and potentially confounding variables are discussed.

Results and Discussion

Firefighters

Firefighters are exposed to potentially carcinogenic combustion and pyrolysis products during the course of their work. A group of 43 firefighters and matched controls were examined for DNA damage that may be related to occupational carcinogen exposures (*2*). Using peripheral blood lymphocytes, we examined *a*) baseline sister chromatid exchange (SCE) frequency, *b*) SCE induction by *in vitro* challenge with mitomycin C, and *c*) PAH–DNA adduct levels. Occupational exposures were determined from histories of firefighting activity, and the presence of confounding factors (e.g., tobacco smoking, charcoal-broiled food consumption, alcohol consumption) were determined by questionnaire (Table 1). Plasma cotinine levels were measured to assess recent exposure to tobacco smoke.

Table 1. Distribution of variables among firefighters and controls.

Variable	Firefighters	Controls
Number of men	43	40
Mean age, years	33.9	34.6
Tobacco users	21	15
Cigarettes/day	22.1	24.9
Charcoal-broiled food (>3 times/month)	29	13
	13	4
Alcohol consumption (daily)	26	21
	8	6

SCE. Mean baseline SCE frequencies were lower in 42 firefighters (8.44 SCE/cell) than in 38 controls (9.23 SCE/cell, $p = 0.02$: Table 2). In addition to firefighting, the effects of other risk factors were evaluated. Smoking was associated with increased baseline SCE. A dose–response relationship between serum cotinine level and baseline SCE was shown ($p = 0.0006$), with an increment of 0.0033 SCE/cell for each nanogram of cotinine per milliliter of serum. Race proved to be a risk factor for elevated SCE levels, with nonwhites showing a higher mean baseline frequency than whites. Two doses of mitomycin C (MMC; 10 and 20 ng/mL) were used as a challenge to determine if prior mutagenic exposure alters *in vitro* response to mutagenic challenges. To remove the uncertain effects of the observed baseline SCE frequency, the MMC inducibility of SCE was calculated by linear regression. When the slopes of the linear regressions were compared, susceptibility to MMC induction was found to be decreased in firefighters who smoked compared to controls who smoked ($p < 0.05$).

PAH–DNA Adducts. Firefighting was not associated with significant risk for PAH–DNA adducts or benzo[*a*]-pyrene diol epoxide (BPDE)–DNA antigenicity as measured by ELISA (*3*) in DNA of nucleated peripheral blood samples (Table 3). However, smoking and alcohol consumption were positively associated with antigenicity ($p = 0.09$ and $p = 0.07$, respectively). No association was found between baseline frequency of SCE and the presence of PAH–DNA adducts.

Hepatoma Patients Treated with Radioimmunoglobulin Therapy

Lymphocytes from patients undergoing radioimmunoglobulin therapy (RIT) were examined for chromosome aberrations expressed immediately upon explant and for chromosome aberrations induced by subsequent challenge with γ radiation after PHA-stimulated proliferation. In addition, *hprt* mutant frequencies were assessed in this same population. Primary hepatoma patients who are candidates for RIT are cyclically injected at approximately 8-week intervals with 30 mCi of ^{131}I-labeled antiferritin. Each cycle results in a whole body radiation dose of approximately 0.3 Gy.

Table 2. Unadjusted baseline sister chromatid exchange frequency among firefighters and controls by two risk factors.

	Firefighters						Controls					
	Smoker		Nonsmoker		Total		Smoker		Nonsmoker		Total	
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
Total	16	9.11 ± 0.93	26	8.02 ± 1.25	42	8.44 ± 1.25	14	9.61 ± 1.89	24	9.01 ± 1.42	38	9.23 ± 1.61
Broiled food (−)	6	9.42 ± 1.42	7	8.53 ± 1.83	13	8.94 ± 1.65	13	9.71 ± 1.93	12	8.47 ± 1.10	25	9.11 ± 1.68
Broiled food (+)	10	8.93 ± 0.48	19	7.84 ± 0.96	29	8.21 ± 0.97	1	8.39	12	9.54 ± 1.55	13	9.46 ± 1.52
Nonwhite	4	9.62 ± 0.23	2	7.91 ± 0.40	6	9.05 ± 0.91	6	9.89 ± 1.94	3	10.3 ± 0.46	9	10.0 ± 1.56
White	12	8.94 ± 1.02	24	8.03 ± 1.30	36	8.34 ± 1.28	8	9.40 ± 1.95	21	8.83 ± 1.95	29	8.89 ± 1.57

Table 3. Polyaromatic hydrocarbon–DNA adduct-positive rates and associated odds ratios among firefighters and controls by risk factors.

	Firefighters			Controls					Total[a]	
	n	Positive[b]	%	*n*	Positive[b]	(%)	OR	95% CI	OR	CI
Total	43	15	(35)	38	13	(34)	1.03	0.41–2.58		
Smoker	17	7	(41)	15	8	(53)	0.61	0.15–2.49	2.44	0.96–6.26
Nonsmoker	26	8	(31)	23	5	(22)	1.60	0.44–5.84		
Nonwhite	6	0	(0)	9	6	(67)	0.04	0.002–0.97	1.33	0.42–4.22
White	37	15	(41)	29	7	(24)	2.14	0.73–6.27		
Alcohol										
Daily	8	6	(75)	6	2	(33)	6.00	0.58–62.1	3.13	0.96–10.2
Others	35	9	(26)	32	11	(34)	0.66	0.23–1.89		
Broiled food (+)	29	7	(24)	12	2	(17)	1.59	0.28–9.07	0.31	0.12–0.82
Times >3/month	13	6	(46)	4	4	(25)	2.57	0.21–31.7	0.77	0.25–2.44
Times <3/month	16	1	(6)	8	1	(13)	0.47	0.03–8.60	0.10	0.02–0.49
Broiled food (−)	14	8	(57)	26	11	(42)	1.82	0.49–6.76		

Abbreviations: OR, odds ratio; CI, confidence interval.
[a]Compared to other dichotomy, e.g., smokers versus nonsmokers, nonwhite versus white.
[b]Number with measurable (positive) adduct levels.

Chromosomal Aberrations. Lymphocytes from individual patients undergoing radiolabeled immunoglobulin therapy were examined both for chromosome aberrations expressed immediately upon explant, or for chromosome aberrations induced by a subsequent challenge of γ-rays after phytohemagglutin-stimulated proliferation (*4*). Despite interpatient variation, there is a strong correlation between levels of chromosome aberrations observed in the initial mitosis after mitogenic stimulation and levels induced by a challenge dose of radiation in replicate cultures after several cell cycles of growth (Fig. 1). These data indicate that even after proliferation, human lymphocytes retain a memory of *in vivo* exposure to ionizing radiation that can be observed by challenge with a clastogenic agent.

Hprt *Gene Mutations.* Gene mutations induced *in vivo* can be measured at the *hprt* locus in human T-lymphocytes. Twenty-eight patients receiving ^{131}I and/or ^{90}Y-labeled antiferritin were studied for mutant frequencies and compared to a control population (*5,6*). Major observations resulting from these studies were *a*) mutant frequencies for patients were increased by an order of magnitude compared to nontreated controls (68.0×10^{-6} versus 6.8×10^{-6}); *b*) there was a good correlation between mutant frequency and the amount of radioactivity given in the first treatment; however, there was a poor correlation between mutant frequency and the total amount of injected isotope over several cycles of treatment; *c*) molecular analyses of mutant clones indicated that radiation induces a higher frequency of gross structural alterations (detectable by Southern blotting) than occur in spontaneous mutations; and *d*) T-cell receptor analysis showed that 84% of mutants arose independently, approximately the same as in seen in control populations.

Cancer Patients Treated with Cyclophosphamide

Cyclophosphamide is a chemotherapeutic alkylating drug commonly used in the treatment of breast cancer, hematopoietic and lymphatic cancers, as well as other malignancies. Cyclophosphamide is a known human carcinogen associated with increased risk for secondary, treatment-associated cancers, most commonly myelogenous leukemias. Major metabolic products associated with cytotoxicity and carcinogenicity are phosphoramide mustard and acrolein. We studied cancer patients treated with cyclophosphamide and pharmacists involved in preparing anticancer drugs (*7,8*).

Acrolein Adducts. Blood specimens were obtained from 27 cancer patients who had primarily breast and hematological malignancies. Twelve patients had been therapeutically treated with a regime that included cyclophosphamide while 15 patients were newly diagnosed, untreated, and served as controls. Acrolein adducts were measured either by ELISA or immunodot blot (IDB). The ELISA assay gave positive results in 4 of 12 treated patients and in 0 of 15 nontreated patients. The IDB assay fared slightly better identifying 6 of 12 treated patients as positive and 0 of 15 untreated controls (Table 4). No clear trends emerged when presence of acrolein-modified DNA was examined as

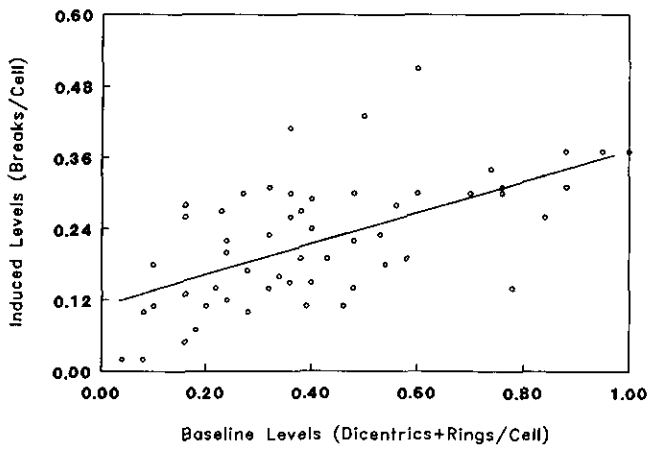

FIGURE 1. Chromosomal radiosensitivity of cells from radioimmunoglobulin therapy patients displayed as a function of levels of chromosome damage sustained *in vivo*. Symmetrical chromosome-type aberrations were scored in first-division metaphases. Parallel cultures from the same individuals to be challenged with radiation *in vitro* were incubated for 72 hr.

Table 4. Acrolein–DNA detection.

Detection method	Number of CP-treated patients (positive/tested)	Number of untreated patients (positive/tested)
ELISA	4/12	0/15
IDB	6/12	0/16

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IDB, immunodot blot; CP, cyclophosphamide.

a function of lifetime cyclophosphamide dose, recent treatment, time since last treatment, treatment regime, or smoking history.

SCE. Blood specimens were obtained from 39 cancer patients; 19 treated with cyclophosphamide and 20 untreated controls. Therapeutically treated patients showed statistically higher baseline SCE frequencies that controls ($p < 0.016$) and returned to baseline in 4–6 weeks after the last treatment dose. Cultured cells from patients treated with cyclophosphamide showed similar *in vitro* responses to challenge doses of phosphoramide mustard as did cells from control patients.

Pharmacists Handling Anticancer Drugs

SCE. Blood specimens were obtained from 34 pharmacy workers who had varying duration of anticancer drug handling (*9*). Each subject completed a questionnaire covering past medical history, family history, current smoking, alcohol, diet, medication, and lifetime occupational history. The mean baseline SCE for the population was 5.19 ± 0.17 and was not correlated with duration of drug handling. However, a strong correlation was demonstrated between inducible SCE values and lifetime duration of drug handling for low-dose phosphoramide challenge (0.1 mg/mL, $r = 0.63$ $p < 0.0001$) and for high-dose phosphoramide challenge (0.25 mg/mL, $r = 0.59$ $p < 0.0001$, Fig. 2).

Women with Benign and Malignant Breast Masses

A prospective, longitudinal study was performed to identity environmental factors that modulate genetic damage in breast cancer patients (*10*). A total of 107 women (49 with breast cancer, 52 with benign breast masses, and 6 normal women) were enrolled.

Hprt *Analysis, Chromosomal Aberrations, and SCE.* Mutant frequency at the *hprt* locus and cloning efficiency of peripheral blood lymphocytes did not differ significantly among the three groups. Mutant frequency increased with age, history of cigarette smoking, and total number of years smoking. Chromosomal aberrations frequencies were similar in lymphocytes of 28 women with benign breast disease (5.3 ± 2.7 aberrations/100 cells) compared to 11 breast cancer patients (5.2 ± 2.1 aberra-

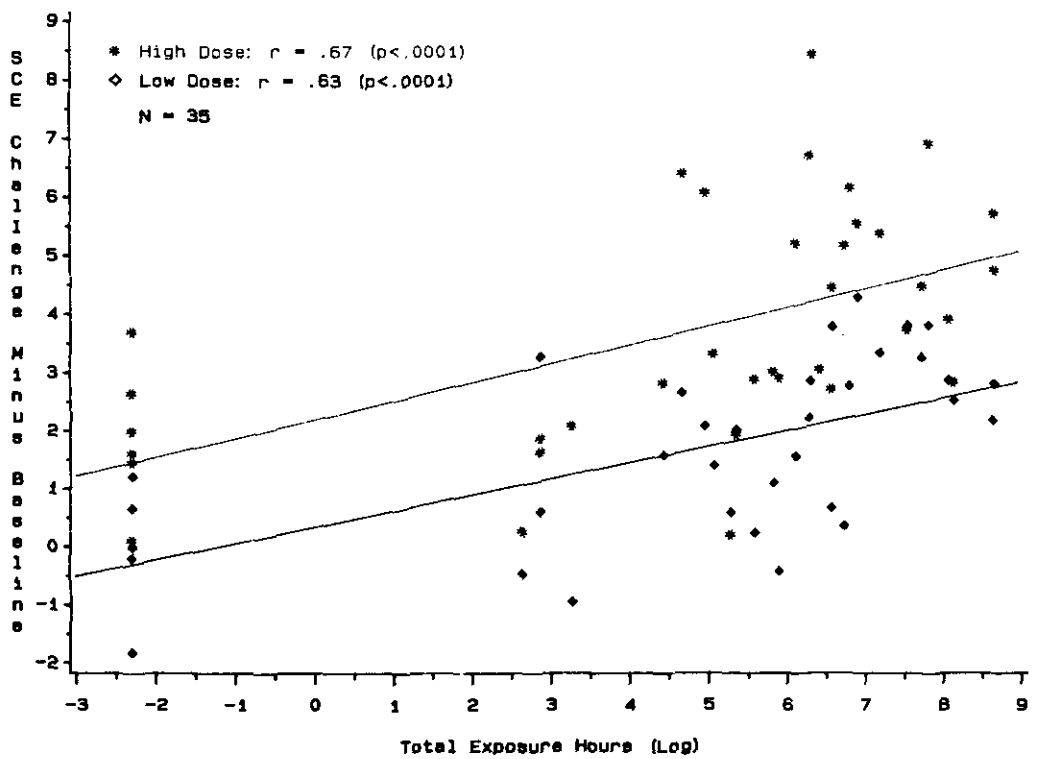

FIGURE 2. Scatter plot and regression of mean sister chromatid exchange (SCE) challenge minus baseline with lifetime antineoplastic drug exposure. (*) High concentration of phosphoramide mustard challenge (0.25 μg/mL) minus baseline SCE; (◆) low-dose phosphoramide mustard challenge (0.1 μg/mL) minus baseline.

tions/100 cells). The mean SCE incidence in lymphocytes from 23 women with benign disease was 9.3 ± 1.6, whereas the rate in cells from 8 cancer patients was 7.5 ± 1.6 (p = 0.01). However, 14 of the former group were smokers, whereas only one of the cancer patients smoked. There was no correlation between the *hprt* mutant frequency and either aberrations or SCE.

American Troops Stationed in Kuwait

We are currently studying a cohort of 60 American troops deployed to Kuwait after Desert Storm. The U.S. government is concerned about potential health hazards associated with pollutants generated by oil-well fires. Blood samples were drawn before deployment, at one time point after 8 weeks in Kuwait, and at another 4 weeks after returning to Germany. SCE and levels of PAH adducts are currently being quantified in this longitudinal study.

Conclusions

We have used batteries of cytogenetic, biochemical, and molecular techniques to monitor diverse human populations who may be at increased risk for cancer because of exposures related to occupation, lifestyle, or medical treatment. Each end point has benefits and disadvantages. For example, induction of SCE is a sensitive end point for certain chemical exposures, especially for exposure to cigarette smoke. However, SCE frequencies respond only marginally to ionizing radiation. In addition, the significance of SCE induction is still not clear. Mutation at the *hprt* locus has proved to be a sensitive indicator of exposure to a variety of agents. On theoretical grounds, however, it is limited to the detection agents that induce point mutations; agents inducing primarily chromosome deletions would not be detected. Additionally, *hprt* mutations do not appear to occur in stem cells because mutant frequencies return to normal levels in 2–3 months after cessation of exposures. Use of antibodies against specific DNA adducts has the advantage of a high level of specificity for particular types of damages. Clearly, such an approach would not be useful for general screening. Also, because DNA adducts are repairable, they are considered as precursors to genetic events. Detection of DNA adducts using antibodies also appears more specific than sensitive (more subject to false negatives than false positives). Chromosomal aberration analysis has been the most widely used of any of the end points. Aberrations have been found to persist in the peripheral blood for for many years, as has been noted for the survivors of Hiroshima and Nagasaki. One must always remember, however, that the cytogenetics events that are generally scored are cytotoxic to the cells that display them and therefore are of no genetic consequence.

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